

Human DNA polymerase α : predicted functional domains and relationships with viral DNA polymerases

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ABSTRACT

The primary sequence of human DNA polymerase α deduced from the full-length cDNA contains regions of striking similarity to sequences in replicative DNA polymerases from *Escherichia coli* phages PRDI and T4, *Bacillus* phage ϕ 19, yeast DNA polymerase I, yeast linear plasmid pGKL1, maize S1 mitochondrial DNA, herpes family viruses, vaccinia virus, and adenovirus. The conservation of these homologous regions across this vast phylogenetic expanse indicates that these prokaryotic and eukaryotic DNA polymerases may all have evolved from a common primordial gene. Based on the sequence analysis and genetic results from yeast and herpes simplex virus studies, these consensus sequences are suggested to define potential sites that subserve essential roles in the DNA polymerase reaction. Two of these conserved regions appear to participate directly in the active site required for substrate deoxynucleotide interaction. One region toward the carboxyl-terminus has the potential to be the DNA interacting domain, whereas a potential DNA primase interaction domain is predicted toward the amino-terminus. The provisional assignment of these domains can be used to identify unique or dissimilar features of functionally homologous catalytic sites in viral DNA polymerases of pathogenetic significance and thereby serve to guide more rational antiviral drug design.

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Key Words: DNA polymerase • primary sequence • sequence similarity • functional domains • herpes simplex virus • deoxynucleotide interaction

EUKARYOTIC GENOMIC DNA REPLICATION is a tightly regulated process that involves the orderly and coordinated interplay of many discrete protein-protein and protein-DNA interactions (1-3). A prerequisite for understanding this complex process is the identification

and characterization of the key protein participants, among which an essential element is the replicative DNA polymerase. DNA polymerase α is generally accepted to be the principal replicative DNA polymerase in eukaryotes (4). Evidence supporting this claim include the following: 1) the expression of DNA polymerase α both at the transcriptional and posttranscriptional levels (5) is regulated, and correlates positively, with the activation of quiescent cells to proliferate; 2) a variety of chemical inhibitors that block DNA synthesis in vivo also inhibit DNA polymerase α activity (4, 6); 3) a temperature-sensitive mutant for DNA synthesis has been identified as a DNA polymerase α mutant (7); 4) monoclonal antibodies that specifically neutralize DNA polymerase α inhibit DNA synthesis in permeabilized cells or when microinjected into nuclei (8-10); 5) polymerase α is required for in vitro SV40 virus DNA replication, which is a model system of eukaryotic cell DNA replication (11-15).

Through nearly 3 decades of intense biochemical studies in many laboratories, the extremely low abundance of this polymerase, its apparent structural complexity, and its extreme lability during conventional biochemical purification have distinguished this enzyme as one of the more problematic in the biochemical literature (4). Several years ago, we established a panel of murine hybridomas that produce monoclonal antibodies of absolute specificity for DNA polymerase α (8). Two immunoaffinity protocols were subsequently developed that enabled the purification of presumptively native forms of polymerase α from human KB cells and the unambiguous identification of the catalytic polypeptide (16, 17). By using the reverse genetic approach, a full-length cDNA clone of the human DNA polymerase α catalytic polypeptide was isolated and characterized (18).

In this review we will summarize and compare the primary protein sequence of human DNA polymerase α with those of several prokaryotic and eukaryotic DNA polymerases of interest. Based on the primary structure comparisons, several regions of similarity are

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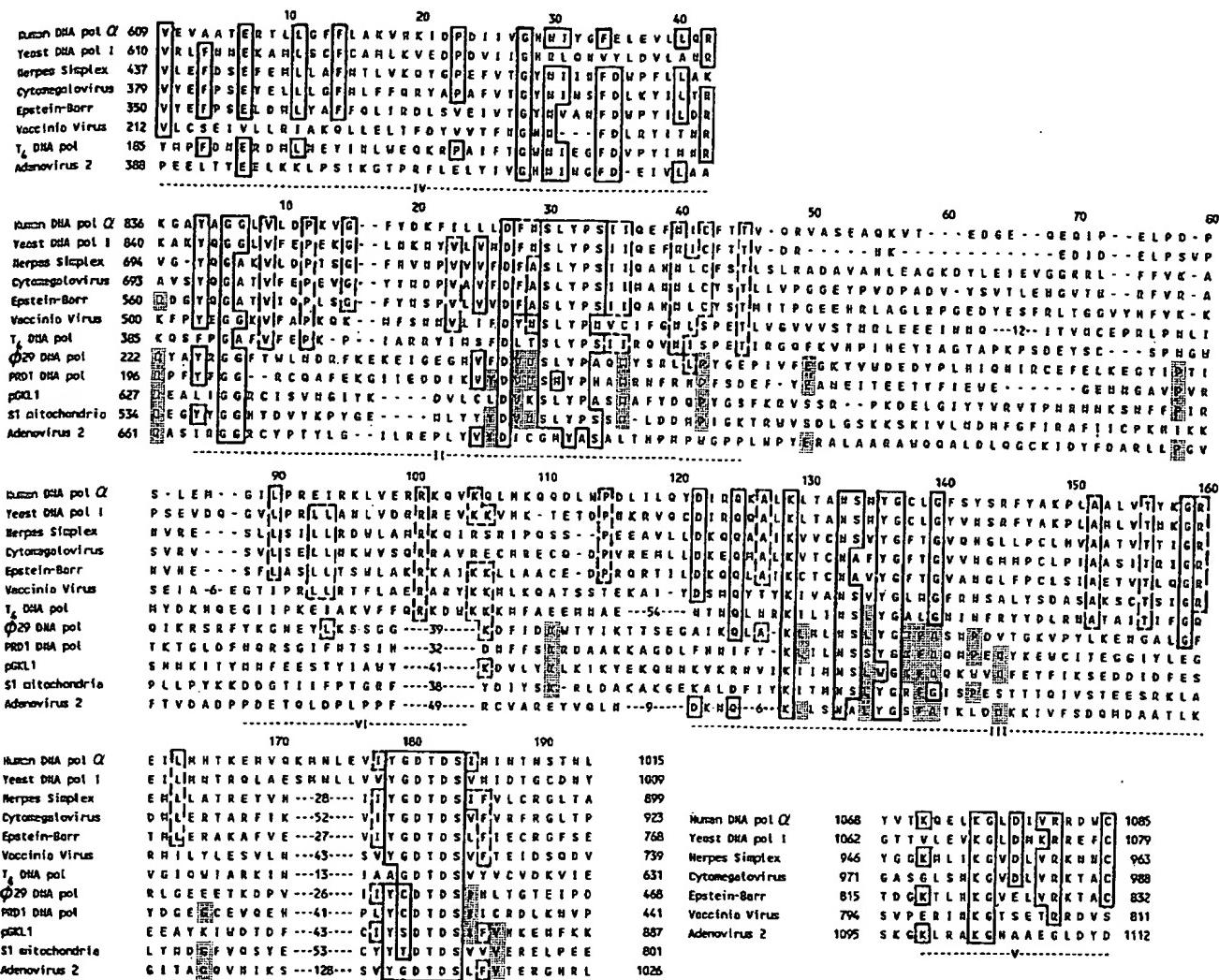


Figure 1. Conserved regions of human DNA polymerase α and other selected DNA polymerases. Amino acid residues 609-1015 from human DNA polymerase α were aligned with amino acid residues derived from other DNA polymerases; identical residues of five or more sequences between human DNA polymerase α and other DNA polymerases are boxed. Gaps are indicated by dashes, and extensive gaps are marked by the number of amino acids contained within the gap. The designated conserved regions are marked by dashed lines under the amino acid residues. Amino acids 998-1005 of human DNA polymerase α are defined as region I; amino acids 839-878 are region II; amino acids 943-984 are region III; amino acids 609-650 are defined as region IV; regions V and VI are amino acids 1075-1081 and 909-926, respectively. Amino acid residues that are uniquely similar among DNA polymerases and use terminal protein priming mechanisms are outlined by stippling (shaded areas); amino acid residues uniquely similar among polymerases that use or are predicted to use DNA primase mechanisms are boxed in dashed lines. Portions of this figure are reprinted, with permission, from ref 18.

detected and are predicted to represent functional catalytic domains of the human enzyme. Provisional assignment of these regions allows an initial assessment of similarities and differences with homologous regions in the replicative polymerases of several classes of viruses of human pathogenetic importance.

PRIMARY PROTEIN SEQUENCE SIMILARITY TO BOTH PROKARYOTIC AND EUKARYOTIC REPLICATIVE DNA POLYMERASES

Comparison of the deduced primary sequence of human DNA polymerase α with known sequences of other DNA polymerases reveals several regions of striking

similarities (18). As shown in Fig. 1, from amino acid number 609 to 1085 of human DNA polymerase α , there are three regions of similarity to the DNA polymerases of *Escherichia coli* phage PRD1 and *Bacillus* phage ϕ 29 and to two potential polymerases (predicted by the polymerase α -like consensus sequences in the ORF) encoded in yeast linear plasmid pGKL1 and maize mitochondrial S1 DNA (19-23); five regions of similarity with *E. coli* phage T4 polymerase, and adenovirus polymerase (24, 25); six regions of similarity with the DNA polymerases of herpes simplex virus (HSV), cytomegalovirus (HCV), Epstein-Barr virus (EBV), and vaccinia virus (26-29); and extensive similarity with yeast DNA polymerase I (18, 30). These regions are designated I to

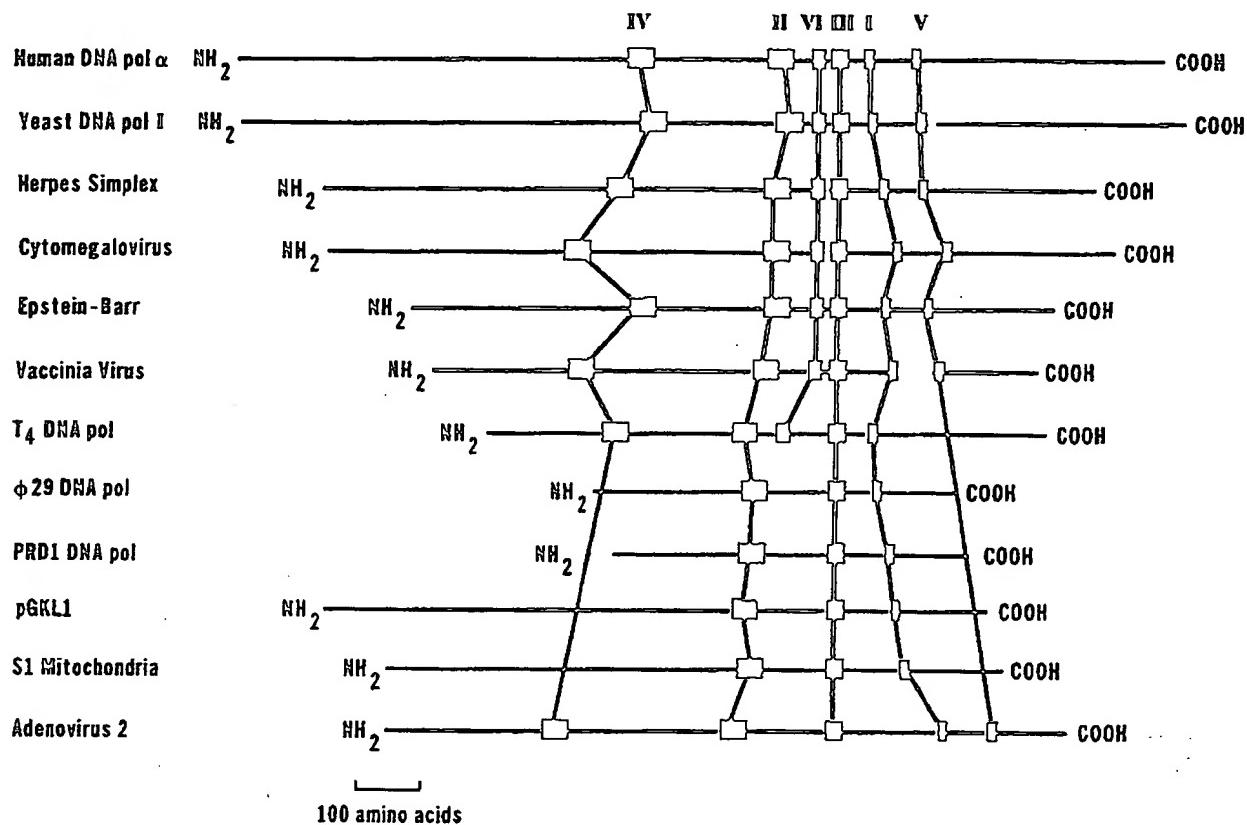


Figure 2. Relative linear spatial arrangements of the conserved regions of DNA polymerases. Each DNA polymerase polypeptide is represented by a straight line with NH_2 and COOH denoting the amino- and carboxyl-terminus, respectively. The red boxes represent similar regions of each DNA polymerase polypeptide are aligned by vertical lines. A portion of this figure is reprinted, with permission, from ref 18.

VI according to their extent of similarity, region I being the most similar and region VI the least (18). The significance of these regions is underscored by their locations on each respective polypeptide (Fig. 2). All six regions exhibit similar linear spatial arrangements on each polymerase polypeptide in the order of IV-II-VI-III-I-V. However, the distances between each of the regions are different.

Human DNA polymerase α and yeast DNA polymerase I are the largest DNA polymerases of this class, each with a molecular mass of about 165 kDa, whereas the *E. coli* phage PRD1 polymerase, which has the smallest molecular mass of 63 kDa, contains only regions I, II, and III (16-19). Regions I, II, and III are present in all of these enzymes and appear to comprise a set of basic core sequences for this DNA polymerase class. Region V is present in all of the eukaryotic polymerases except for the two potential DNA polymerases encrypted in yeast plasmid pGKL1 and maize S1 mitochondrial DNA. Adenovirus DNA polymerase and the two potential DNA polymerases lack region VI; coliphage T4 DNA polymerase is the only prokaryotic polymerase that has both regions IV and VI (Fig. 2). Sequence comparison of human DNA polymerase α with other eukaryotic DNA polymerases, such as DNA polymerase β , terminal transferase, or reverse transcript-

tase (31, 32), and other prokaryotic DNA polymerases, such as *E. coli* DNA polymerases I and III, reveals no significant similarity (33, 34).

Southern hybridization of two restriction fragments of human DNA polymerase α cDNA that contain five of the six consensus sequences with genomic DNA samples from the calf, rodent, amphibian, insect, and plant demonstrates the presence of these consensus sequences in all of the examined vertebrates and invertebrates, but not in plants (35).

PREDICTED FUNCTIONAL DOMAINS

In the process of DNA replication, DNA polymerase α is involved with other replication proteins in an ordered sequence of binding reactions with DNA template, primer, and dNTPs (1-3). There are essentially two different modes by which DNA synthesis is initiated. *E. coli* phage PRD1, *Bacillus* phage ϕ 29, and adenovirus replicate their DNA by a protein priming mechanism (1, 2, 20), whereas all other eukaryotic organisms that have been studied, including such DNA viruses as HSV, HCV, EBV, and vaccinia virus, are thought to initiate DNA synthesis not by protein priming but by oligoribonucleotide priming mechanisms. In at least some instances, the requisite oligonucleotide primers

are synthesized by DNA primase. The common finding of a tightly associated DNA primase in DNA polymerase α fractions highly purified from a variety of eukaryotic organisms suggests that a potential DNA primase interaction domain is present on the polymerase α molecule. The polymerase α polypeptide must also contain DNA interaction domain or domains for template-primer recognition and one or more dNTP interaction domains. By comparative analysis of the primary protein sequence of human DNA polymerase α with those of other DNA polymerases, the regions responsible for these primase, DNA, and dNTP interactions can be provisionally identified.

Predicted DNA primase interaction domain

The gene of yeast DNA polymerase I was recently sequenced (30). A temperature-sensitive mutant of yeast polymerase I has been obtained by *in vitro* mutagenesis (36), which, at nonpermissive temperature, can complete one round of cell division and DNA replication before it arrests. Biochemical characterization indicates that the mutation leads to a conformational change at elevated temperature that affects the stability

of the DNA primase-DNA polymerase I complex (30, 36). Sequence comparison of human DNA polymerase α and yeast polymerase I demonstrates an overall 31% sequence similarity between these two proteins (Fig. 3). Analysis of the temperature-sensitive mutant of yeast polymerase I identified a point mutation that confers the temperature-sensitive phenotype and maps to consensus region IV as a single amino acid substitution of glycine at codon 493 to positively charged arginine. This region of yeast polymerase I exhibits near-perfect homology with human DNA polymerase α (Fig. 3). Although such a single amino acid change might cause an indirect charge effect on polypeptide folding, it is attractive to consider that the mutation defines a domain that is responsible for DNA primase/DNA polymerase interaction.

Comparison of the primary sequences of this class of DNA polymerases also reveals the presence of several sequences that are unique to those enzymes that replicate DNA by a protein priming mechanism (Fig. 1). It is interesting that these unique sequences are also present in the two potential polymerases, i.e., those from yeast pGKL1 and maize S1 mitochondria. The implication of these sequences in the priming protein

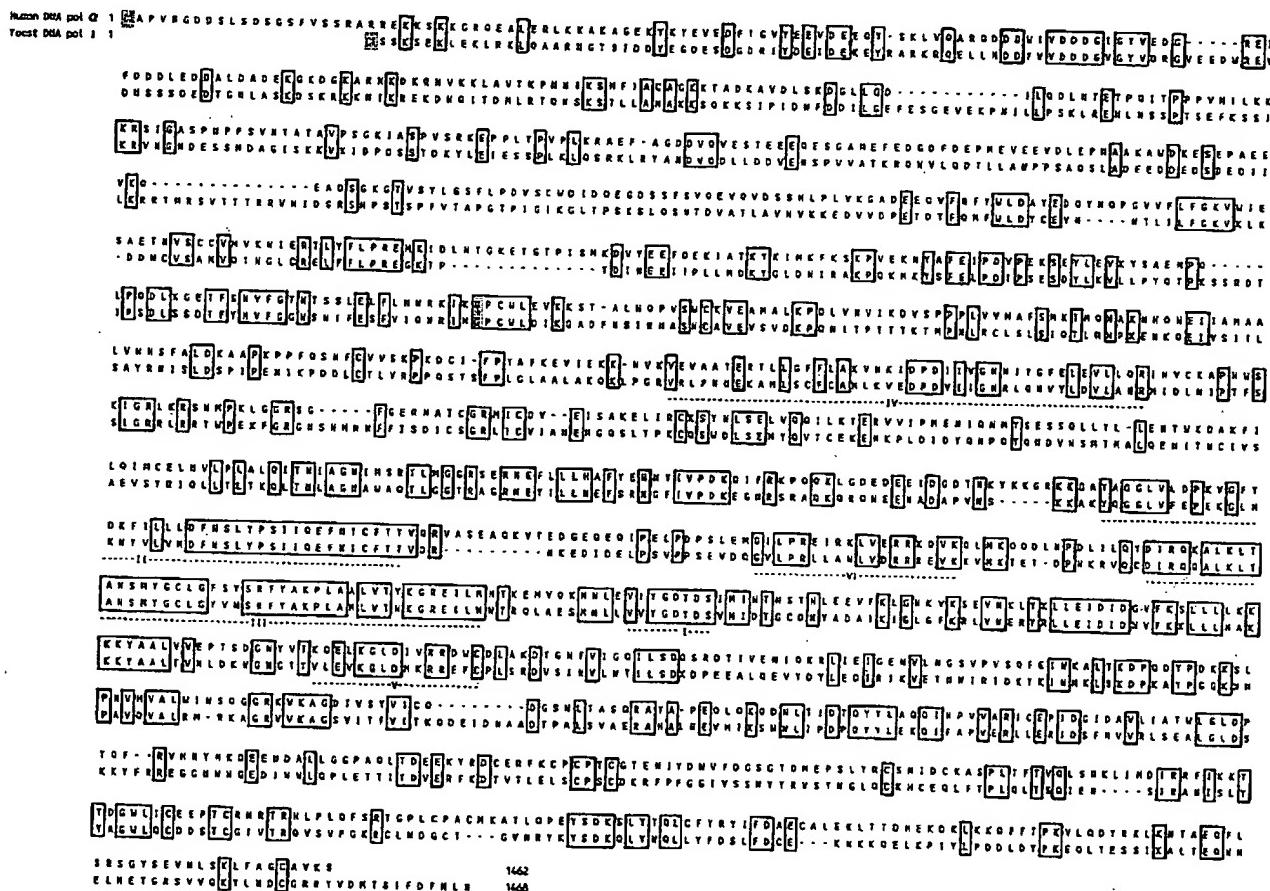


Figure 3. Primary protein sequence comparison of human DNA polymerase α and yeast DNA polymerase I. Identical amino acid residues are boxed, and the six consensus regions are underlined by dashed lines. The first methionine residues are stippled, as is the glycine₄₉₃ of yeast polymerase I, the site of a yeast pol I temperature-sensitive mutation with altered stability of DNA primase association. Human DNA polymerase α sequence is reprinted, with permission, from ref 18, and yeast DNA polymerase I sequence, with permission, from ref 30.

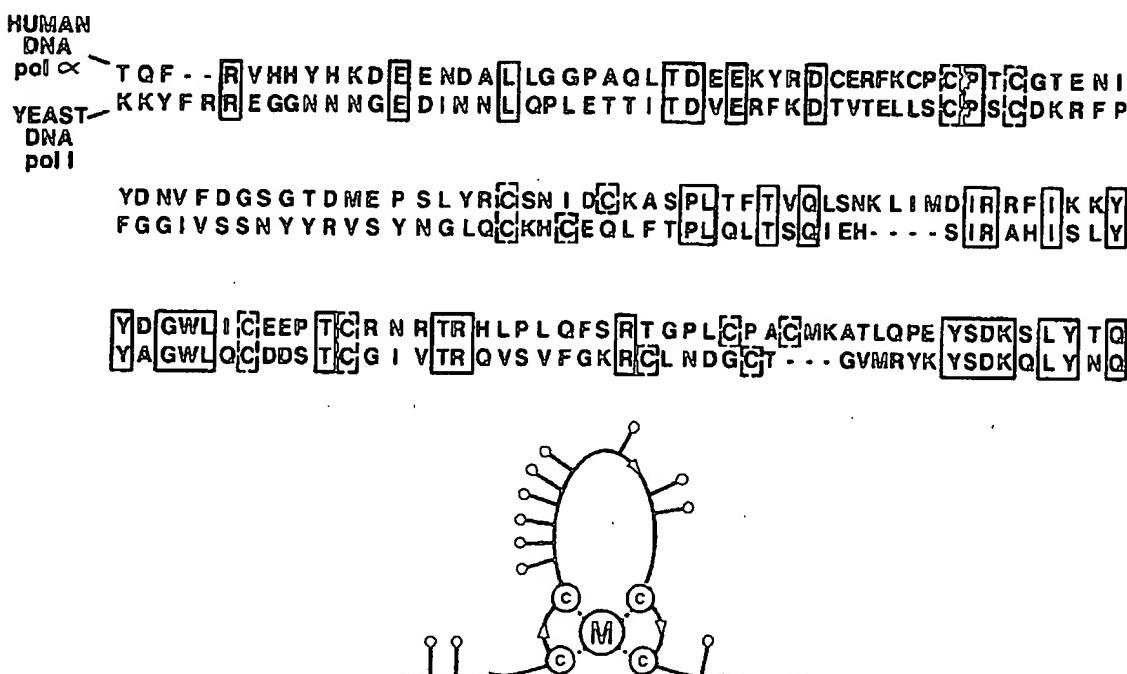


Figure 4. Amino acid sequence of possible DNA interaction region of human DNA polymerase α and yeast DNA polymerase I. Human DNA polymerase α amino acids 1244 to 1391 and yeast pol I amino acids 1246 to 1389 are depicted. Identical amino acids are boxed in solid lines, and cysteines are boxed in dashed lines. A schematic potential DNA-binding loop is illustrated with most probable DNA-binding side chains marked as black circles. The cysteine residues potentially involved in metal ion coordination are depicted in red. Human DNA polymerase α sequence is derived from ref 18 and yeast DNA polymerase I sequence from ref 30, with permission.

interaction remains to be verified by future mutagenesis studies.

DNA interaction domain

Sequence comparison of human DNA polymerase α and yeast DNA polymerase I reveals a cysteine-rich region toward the carboxyl-terminus in both DNA polymerases (Fig. 4). From amino acid number 1244 to 1391 of human DNA polymerase α , there is a region potentially able to form a DNA binding motif (Zn-finger) with an extended protein loop containing amino acids with side chains having the potential to interact with the phosphate backbone of DNA (37-39). Figure 4 presents a schematic depiction of this putative DNA interaction domain.

Deoxynucleotide interaction domains

The presence of the three consensus sequences (regions I, II, and III) in the DNA polymerases of bacteriophages, DNA viruses, unicellular fungi, invertebrates, and vertebrates suggests that these sequences subserve essential functions in polymerase catalysis (18, 35). Sequence comparison of human DNA polymerase α and the HSV DNA polymerase (HSV pol) reveals homology in all six conserved regions (18), with 87.5% in region I; 60% in region II; 47% in region III; 26% in region IV; 57% in region V; and 10.5% in region VI (Fig. 1 and Fig. 5). By marker rescue and marker transfer ex-

periments, many HSV mutants have been identified that contain mutations in the DNA polymerase gene; some of these mutations confer altered sensitivity to either aphidicolin (Aph) or antiviral drugs such as acyclovir (ACV), bromovinyldeoxyuridine (BVdU), ganciclovir (DHPG), vidarabine (araA), and phosphonoacetic acid (PAA) (28, 29, 40-42). Mutations that confer altered sensitivity to these drugs (all of which are either analogs of dNTPs or pyrophosphate, or competitive inhibitors, such as aphidicolin) are potential markers of the dNTP and PP_i binding site or sites.

Because any single mutation might affect substrate or antiviral drug recognition simply by an indirect charge effect, it is essential to examine a large number of mutants. Sequence analysis of many of these mutants from three strains of virus has revealed that a majority of the mutations are single amino acid substitutions within consensus regions II and III (28, 40, 42) (Fig. 5). Thus, it is reasonable to predict that these regions are sites that are directly involved in dNTP binding or pyrophosphate hydrolysis (40). However, two mutations have recently been identified that do not reside within regions II or III. One, in region V, has a single amino acid change of asparagine₉₆₁ to lysine, which confers altered sensitivity to aph and DHPG; the corresponding residue at this site in human polymerase α is aspartate (18, 40). Another mutation, PAA'C, localized between regions VI and III, confers resistance to phosphonoacetic acid and has a single amino acid substitution of proline₉₇ to threonine; interestingly, human

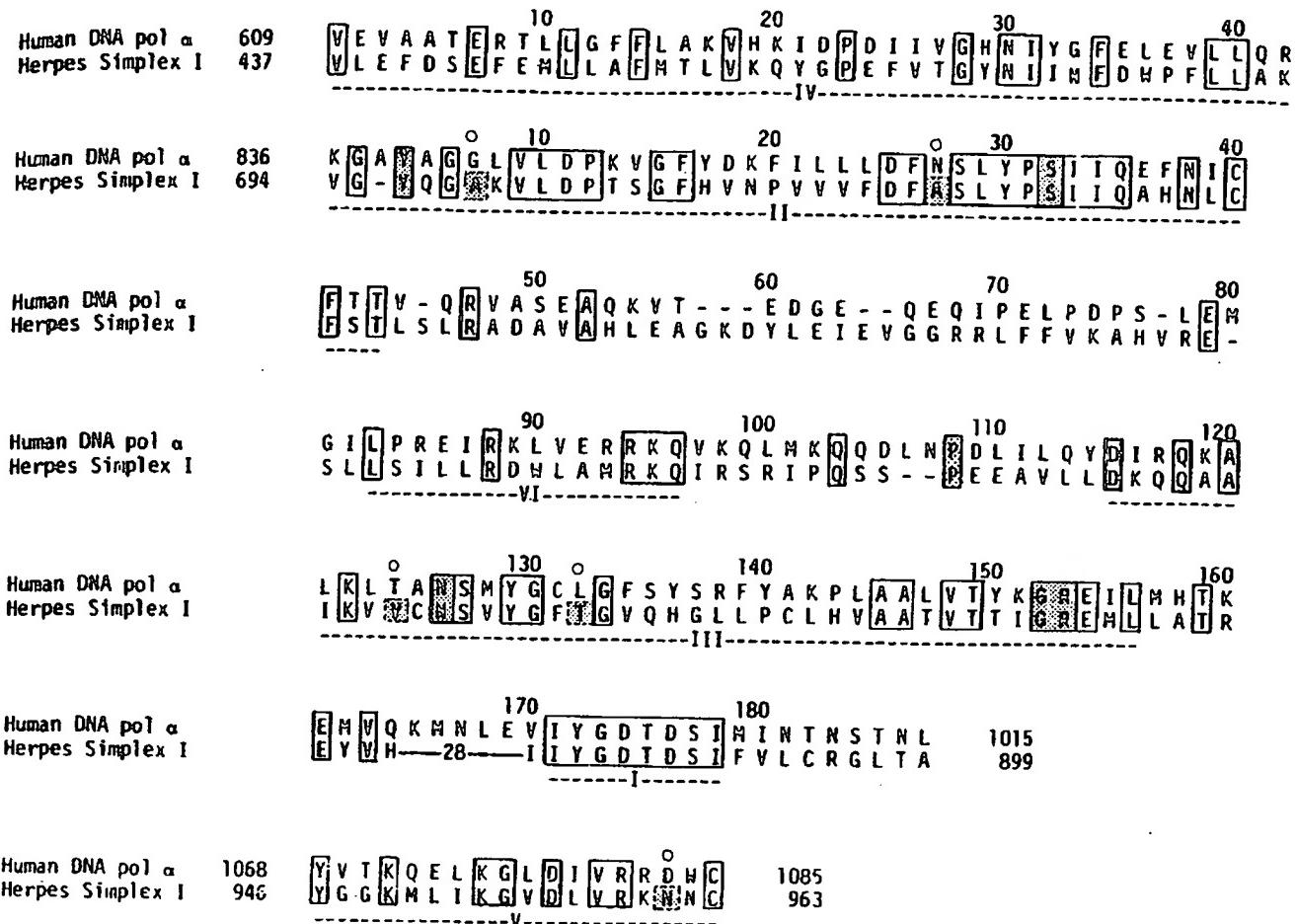


Figure 5. Conserved sequences between human DNA polymerase α and herpes simplex virus I DNA polymerase, with predicted dNTP interaction domains. The six conserved regions are aligned, and identical amino acid residues are boxed. Amino acids that are identical to herpes simplex virus mutants with single amino acid substitutions are boxed with solid lines and shaded. Herpes virus polymerase mutations at positions nonidentical to DNA polymerase α are shown by designating the substituted amino acid in a shaded box with dashed lines and the corresponding polymerase α residue with a large black dot. Reprinted from ref 18 and derived from refs 27 and 40, with permission.

DNA polymerase α has an identical proline residue at this position (40). Figure 6 summarizes these predicted functional domains.

NONCONSERVED REGIONS OF VIRAL REPLICATIVE ENZYMES THAT ARE INVOLVED IN DRUG RECOGNITION

Several mutants of herpes simplex polymerase with altered drug sensitivity have amino acid substitutions at loci that are dissimilar in sequence to human DNA polymerase α . Recently, Gibbs et al. (40) characterized two mutants derived from wild-type HSV type I, strain KOS, with altered drug sensitivity phenotypes that were mapped to the HSV *pol* gene in a unique region A, from amino acid number 565 to 637 of HSV *pol*. This region, as defined by two mutations (*tSD9*, glutamic₅₉₇ to lysine; *PFA^r2*, alanine₆₀₅ to valine) has sequence similarity only to other viral DNA polymerases that exhibit sensitivity to certain antiviral drugs.

Remarkably, this region is not present in the human cell DNA polymerase α sequence. In addition to these changes in region A, four other mutations of HSV *pol* within regions II and III exhibit substitutions at amino acid residues that are not identical in human polymerase α .

The further characterization of these HSV *pol* mutants in reference to the predicted functional domains of human DNA polymerase α should comprise a particularly attractive approach to the design of specifically targeted antiviral drugs.

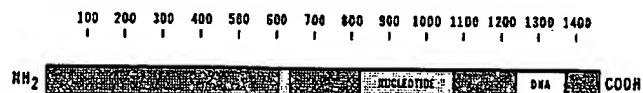


Figure 6. Schematic representation of the putative functional domains of human DNA polymerase α . Reprinted and modified from ref 18, with permission.

FUTURE PERSPECTIVES

The genetic information content of chromosomal DNA must be copied accurately by DNA polymerases for faithful transmission from one generation to the next. How eukaryotes accomplish error-free DNA replication with a principal replicative DNA polymerase, pol α , that does not have an intrinsic exonuclease activity for redactory function remains an intriguing question. In vitro steady-state kinetics studies have demonstrated that polymerase α interacts with its substrates by a rigidly ordered terreactant sequential mechanism and exhibits respectable fidelity and modest processivity in the absence of any exogenous or accessory proteins (17, 43, 44). Full explication of the molecular basis of this complex DNA polymerization reaction remains a formidable challenge. The identification of six consensus regions in human DNA polymerase α that are present in both prokaryotic and eukaryotic replicative DNA polymerases argues that these enzymes may comprise a family that has arisen from a common ancestral gene (18, 35) and that the homologous regions have been conserved to accomplish the elementary steps of the DNA polymerization reaction.

The predicted functional domains for DNA interaction, primase interaction and dNTP interaction should soon be testable by assessing the functional expression of recombinant DNA polymerases designed by site-specific mutagenesis. Careful examination of the kinetic parameters of functional mutants of DNA polymerase α in comparison with those of the well-characterized wild-type polymerase should shed light on how this large and complicated protein molecule recognizes and interacts with its several substrates to accomplish accurate DNA replication. In addition, the identification of domain or domains required for interaction with other accessory DNA replication proteins should greatly advance our understanding of the details of eukaryotic DNA replication in vitro and in vivo.

Extensive studies of herpes simplex DNA polymerase mutants with altered sensitivity to antiviral drugs have failed to define any specific, single region on the HSV polymerase molecule as the sole binding site for these drugs or substrates (40). This suggests that dNTP interacting site or sites may require interactions among several separate regions on the polymerase molecule, a hypothesis that can be examined either by isolation of suppressor mutants or by expression of appropriately constructed recombinant herpes DNA polymerases, followed by physical studies. The expression of enzymatically active recombinant adenovirus DNA polymerase from cloned DNA was recently accomplished (45, 46). Site-specific mutagenesis in region I was attempted but resulted in an inactive enzyme (J. A. Engler, personal communication). This result, together with the failure to date to isolate mutants in region I of the HSV DNA polymerase, suggests that region I may be particularly critical for DNA polymerization and viral viability.

Mutagenesis studies of expressed recombinant DNA polymerases from human cells and viruses, combined

with examination of physicochemical properties and molecular structure, should ultimately permit precise anatomic delineation and functional assignment to each of these regions and thereby facilitate the development of a comprehensive molecular understanding of the DNA polymerization reaction. [F]

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VOLUME 5 NUMBER 1

CONTENTS

Editorial	1
Public Affairs	3

Reviews

Therapeutic opportunities involving cellular oncogenes: novel approaches fostered by biotechnology. <i>B. E. Huber</i>	5
Human DNA polymerase α : predicted functional domains and relationships with viral DNA polymerases. <i>T. S.-F. Wang, S. W. Wong, and D. Korn</i>	14
AIDS: a syndrome of immune dysregulation, dysfunction, and deficiency. <i>A. S. Edelman and S. Zolla-Pazner</i>	22
Endothelium-derived nitric oxide: actions and properties. <i>L. J. Ignarro</i>	31

Research Communications

Nuclear disintegration of cells by killer B lymphocytes from tumor-bearing mice. <i>D. M. Lopez, B. B. Blomberg, R. R. Padmanabhan, and L. Y. W. Bourguignon</i>	37
Guanine nucleotide-dependent, pertussis toxin-insensitive regulation of phosphoinositide turnover by bradykinin in bovine pulmonary artery endothelial cells. <i>T. A. Vojno-Yasenetskaya, V. A. Tkachuk, E. G. Cheknyova, M. P. Panchenko, G. Y. Grigorian, R. J. Vavrek, J. M. Stewart, and U. S. Ryan</i>	44
Heterogeneity of pH in the aqueous cytoplasm of renal proximal tubule cells. <i>T. Y. Aw and D. P. Jones</i>	52
Cytosolic-free Ca^{2+} and cell killing in hepatoma 1c1c7 cells exposed to chemical anoxia. <i>P. Nicotera, H. Thor, and S. Orrenius</i>	59
Occurrence of platelet-activating factor (PAF) in normal rat stomach and alteration of PAF level by water immersion stress. <i>J. Sugatani, K. Fujimura, M. Miwa, T. Mizuno, Y. Sameshima, and K. Saito</i>	65
High-yield trapping of EGF-induced receptor dimers by chemical cross-linking. <i>B. O. Fanger, J. E. Stephens, and J. V. Staros</i>	71

Calendar	76
---------------------------	----

Book Reviews	84
-------------------------------	----

Books	87
------------------------	----

Federation News	88
----------------------------------	----

Wellcome Visiting Professorships; AAI member awarded Griffuel prize; LSRO study on anemia in childbearing women; FASEB 73rd Annual Meeting—news and previews

Special Feature: This Is FASEB. <i>Robert W. Krauss</i>	94
--	----

News	101
-----------------------	-----

Malaria vaccine testing; kidney stones; health funding; grants in biological sciences

New Products and Literature	102
--	-----

Information for Authors	105
--	-----

Employment Opportunities	110
---	-----

Reviews

Transgenic mammals and biotechnology. H. Westphal

Two hypotheses on the feedback regulation of pancreatic enzyme secretion. T. Fushiki and K. Iwai

Role of the little brain in the gut in water and electrolyte homeostasis.

H. J. Cooke

Renal α -adrenergic receptor alterations: a cause of essential hypertension.

M. M. Michel, P. A. Insel, and O. E. Brodbeck

Neutral endopeptides 24.11 (enkephalinase) and related regulators of peptide hormones. E. G. Erdös and R. A. Skidgel

Research Communications

Enzyme replacement therapy in fibroblasts from a patient with cholesterolemia storage disease. M. J. Poznansky, S. K. Hutchison, and P. J. Davis

Danazol therapy renders red cells resistant to osmotic lysis. Y. S. Ahn, C. I. Kim, R. Mylvaganam, L. F. Fernandez, J. D. Temple, M. L. Cayer, and W. J. Harrington

Elimination of permeability mutants from selections for drug resistance in mammalian cells. M. J. Schibler, S. B. Barlow, and F. Cabral Calorie restriction delays age-dependent loss in androgen responsiveness of the rat liver. B. Chatterjee, G. Fernandes, B. P. Yu, C. Song, J. M. Kim, W. Demyan, and A. K. Roy

Hepatocyte function and extracellular matrix geometry: long-term culture in a sandwich configuration. J. C. Y. Dunn, M. L. Yarmush, H. G. Koebe, and R. G. Tompkins

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